CDMRP
Department of Defense

Breast Cancer Research Program





U.S. Army Medical Research and Materiel Command

Congressionally Directed Medical Research Programs

HISTORY

The Office of the Congressionally Directed Medical Research Programs (CDMRP) was created in 1992 from a powerful grassroots effort led by the breast cancer advocacy community that resulted in a congressional appropriation of funds for breast cancer research.

This initiated a unique partnership among the public, Congress, and the military. Since then, the CDMRP has grown to encompass multiple targeted programs and has been responsible for managing over $10.8 billion since its inception through fiscal year 2016 (FY16). Funds for the CDMRP are added to the Department of Defense (DoD) budget, in which support for individual programs, such as the Breast Cancer Research Program (BCRP), is allocated via specific guidance from Congress.

APPLICATION REVIEW PROCESS

The CDMRP uses a two-tier review process for application evaluation, with both tiers involving dynamic interaction among scientists and disease survivors (consumers). The first tier of evaluation is a scientific peer review of applications measured against established criteria for determining scientific merit. The second tier is a programmatic review conducted by the Programmatic Panel, which is composed of leading scientists, clinicians, and consumers. The Programmatic Panel compares applications to each other and makes recommendations for funding based on scientific merit, potential impact, adherence to the intent of the award mechanism, relevance to program goals, and portfolio composition.

Breast Cancer Research Program

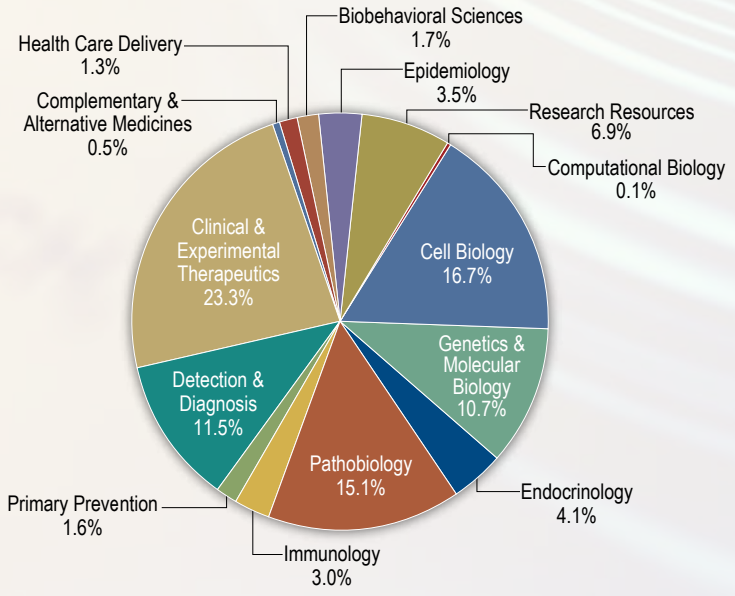
VISION

To end breast cancer by funding innovative, high-impact research through a partnership of scientists and consumers

ABOUT THE PROGRAM

The BCRP plays a leading role in the fight against breast cancer through its innovative approaches and its focus on research that will bring an end to this disease. The BCRP was established in 1992 as a result of the dedicated efforts of breast cancer advocates. Their continued efforts, in concert with the program's accomplishments, have resulted in more than $3.2 billion in congressional appropriations through FY16. The BCRP enables researchers to propose their best, innovative ideas that address the urgent need to end breast cancer. Scientists are challenged to pursue high-risk, high-reward research, explore new paradigms that could lead to critical discoveries, and make an unprecedented impact on breast cancer. The BCRP also promotes synergistic collaborations across disciplines and integrates scientists and consumers in unique and meaningful research partnerships.

FY93–FY15 BCRP Portfolio

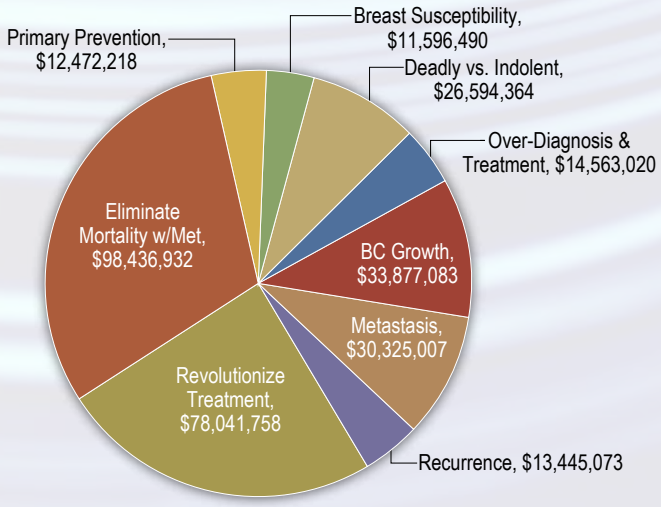


BCRP Overarching Challenges

Considering the current breast cancer landscape and the BCRP's vision to end breast cancer, the BCRP requires applications to address at least one of the following overarching challenges:

- Prevent breast cancer (primary prevention)
- Identify determinants of breast cancer initiation, risk, or susceptibility
- Distinguish deadly from indolent breast cancers
- Conquer the problems of overdiagnosis and overtreatment
- Identify what drives breast cancer growth; determine how to stop it
- Identify why some breast cancers become metastatic
- Determine why/how breast cancer cells lie dormant for years and then re-emerge (recurrence); determine how to prevent recurrence
- Revolutionize treatment regimens by replacing them with ones that are more effective and less toxic
- Eliminate the mortality associated with metastatic breast cancer

FY13–FY15 BCRP Funding Invested by Overarching Challenges



The Breast Cancer Landscape

The BCRP has prepared an overview of the breast cancer landscape, covering the topics most pertinent to the program's vision of ending breast cancer.

Some key points from *The Breast Cancer Landscape:*

- Worldwide, breast cancer accounts for nearly a quarter of all cancers in women. In 2012, there were 522,000 breast cancer deaths globally.
- Evidence attributes the majority of breast cancers, not to one single factor, but to various physical, environmental, and genetic factors.
- Most risk factors are not modifiable, including age, family history, BRCA mutation status, and breast density. Potentially modifiable risk factors, such as obesity, alcohol consumption, smoking, and exercise, are weakly to moderately associated with breast cancer risk.
- An estimated 20-30% of women diagnosed with invasive breast cancer will have a recurrence.
- The rate of metastatic breast cancer at initial diagnosis in the United States has not changed since 1975.
- Treatments to permanently eradicate metastasis do not exist. There is no cure once metastatic disease has occurred.

Read *The Breast Cancer Landscape* at http://cdmrp.army.mil/bcrp/pdfs/bc_landscape.pdf.

Strategic Partnerships

The BCRP is widely recognized as a model biomedical research program, and meaningful partnerships have been the foundation of the program's successes from the very beginning. Through this program, the combined efforts of many dedicated individuals foster unique opportunities in breast cancer research. All aspects of the BCRP, including setting program priorities, designing funding opportunities, evaluating and recommending applications for funding, and conducting high-impact research, integrate the expertise of scientists with the perspectives of consumers. Utilizing these innovative approaches is a proven and effective way to support and advance research that has the potential to make a meaningful impact and contribute to the program's vision of ending breast cancer.

"The DoD BCRP is driven by a singular vision, to end breast cancer. While some progress has been made, breast cancer remains the most commonly diagnosed cancer among women, with an estimated 1.7 million women diagnosed each year worldwide. This complex problem requires multi-faceted, innovative solutions, and through a unique partnership between scientists and consumer advocates, the BCRP supports research with a clear potential to have a high impact. I deeply value and appreciate the opportunity to contribute to the realization of the BCRP's vision through serving on the Programmatic Panel."

Chris Li, Fred Hutchinson Cancer Research Center
FY17 Programmatic Panel Chair

"I have been treated with Herceptin® since my initial diagnosis with metastatic breast cancer. The DoD funded early work on Herceptin and thus benefitted me as an active-duty Service Member, and now as a Veteran. I and other breast cancer survivors are anxious and frustrated that no cure exists for metastatic breast cancer, and yet, I walked away from the peer review experience feeling assured that researchers are indeed working hard and trying to find solutions to end this disease."

Sheila Johnson-Glover, E-8/SMSgt (Ret), U.S. Air Force
St. Louis Breast Cancer Coalition

"The BCRP is a unique and innovative forum for elevating research beyond incremental advancements. It is indispensable to the breast cancer community. By serving as a voting member of scientific review panels, I have been able to bring the perspective of survivors to the table. Advocate involvement in the program is essential and ensures that the voices of survivors are heard. The experience of collaborating with scientists to influence cutting-edge research and representing those affected by breast cancer has been priceless."

Joan Mancuso, SHARE

Scientists and consumers working together to end breast cancer

"Being an Era of Hope Scholar has enabled me to really broaden my research program aimed at developing therapies to prevent and combat metastases. As a result, early in my career, I have been able to recruit top research talent and pursue directions and take chances that I never would have been able to otherwise. The scholarship allowed me to nucleate a team of scientific experts and patient advocates, all geared around solving one problem. I have no doubt that the resulting interactions, particular those with our patient advocates Teri and Rebecca, are going to push our research somewhere special."

Cyrus Ghajar, Fred Hutchinson Cancer Research Center

"Beyond my personal medical care provided by the military, it means so much to me that the DoD is also providing for military families by funding such important research on breast cancer and metastasis. I know that the program has resulted in current treatments like Herceptin®, and there are many others in the pipeline. Maybe one day, through the research funded by the DoD, more lives will be saved from this deadly disease. Maybe one of those saved will be me. I'm hoping to be a miracle by making it to 5 years with no active disease. I am well aware that the research might not save me, but that is not going to stop me from fighting."

Alexis Rhoads, Annie Appleseed Project

"We work very closely with consumer advocates during all phases of our projects - from initial development to lab meeting presentations to attendance at our summer weekend lab retreat to manuscript preparation. Over the past several years, we have not only developed effective collaborations, but also established lasting friendships."

Sandra McAllister, Harvard Medical School and Brigham and Women's Hospital

"Being involved in the Breast Cancer Research Program is truly an exceptional experience because you are sitting in the room with scientists, with researchers, with clinicians, all of whom are working together to try and come to the right decision on funding. And you are part of that process, and it's a very sobering, very exciting, and very responsible position."

Amy Bonoff*, SHARE

*Amy Bonoff passed away on December 17, 2015.

In the Clinical Pipeline

The BCRP has invested in the discovery and development of multiple therapies and diagnostic tools, many of which have continued to advance through the clinical pipeline. The current clinical testing phase of products that have been supported by the BCRP at some point in their clinical development are shown here, with additional details available on pages 7-10.

BCRP-funded *Current phase supported by other sources* *Previous phase supported by other sources*

Development Area	Agent/Technique	Pre-IND*	Phase I/II	Phase III
Vaccines	E75 Her2-derived Peptide Vaccine (NeuVax™)	BCRP	BCRP	Current
	HER2 Peptide-Based Vaccine		BCRP	
	STEMVAC		BCRP	
	Mammoglobin cDNA Vaccine		BCRP	
	Folate Receptor Alpha Vaccines		BCRP	
Immunotherapies	HER2 Bi-Armed Activated T Cells	BCRP	Current	
	TRC105	BCRP	Current	
	Tumor Antigen-Targeted T-Cell Therapy for Metastatic Breast Cancer	BCRP	Current	
Novel Techniques in Treatment	HER2-Targeted Drug Delivery	BCRP	Current	
	Targeted HER2 Radiotracer	BCRP	BCRP	
	Polycationic Peptides for Fluorescence-Guided Surgery	BCRP	Current	
Therapeutics	Center of Excellence for the Eradication of Brain Metastasis	BCRP		
	IDO Inhibitor	BCRP	Current	
	Pembrolizumab and Tremelimumab for Treatment of Oligometastasis	BCRP	BCRP	
	Combining Aromatase and Src Inhibitors	BCRP	Current	
	5-Fluoro-2'deoxycytidine (FdCyd)	BCRP	BCRP	
	Anti-Androgen Therapy (Enzalutamide)	BCRP	BCRP	
	Meclofenamate for Brain Metastasis	BCRP	BCRP	
	Aspirin as Adjuvant Therapy for Node-Positive Breast Cancer			BCRP
Diagnostics	Intraductal Techniques	BCRP		
	Skp2 Oncogene	BCRP	Previous	Current

*Investigational New Drug (IND)

Vaccines:

- **HER2 Peptide-Based Vaccine**

  Mary (Nora) L. Disis

  The human epidermal growth factor receptor 2 (HER2) intercellular domain peptide-based vaccine is designed to treat breast cancer by stimulating the immune destruction of remaining cancer cells after primary cancer therapy. The vaccine was evaluated in a Phase II clinical trial in stage III and IV HER2+ patients, resulting in improved survival in patients with advanced stage HER2+ breast cancer when administered early in the course of treatment. The vaccine has been licensed by EpiThany fur further investigation.

- **Folate Receptor Alpha Vaccines**

  Keith Knutson, Edith Perez

  The folate receptor alpha has been shown to be highly expressed in triple-negative breast cancers (TNBC). The BCRP is supporting a Phase II clinical trial (anticipated to begin in February 2017) to determine whether a folate receptor alpha vaccine can prevent or delay disease recurrence in patients with TNBC. Drs. Knutson and Perez have shown the safety and immunogenicity of the vaccine in a Phase I trial. A safety profile of vaccination will also be determined from this Phase II trial, along with markers of disease protection.

- **STEMVAC**

  Mary (Nora) L. Disis

  Dr. Disis has successfully created a multi-antigen vaccine, STEMVAC, comprised of Th1 epitopes derived from five breast cancer stem cell/EMT immunogenic proteins. The vaccine has been shown to be safe and to inhibit tumor growth in mouse models of breast cancer, and Dr. Disis has recently started a Phase I clinical trial in patients with HER2-negative, advanced stage breast cancer. This trial will text toxicity as well as how the vaccine impacts development of immunologic memory. If the vaccine proves safe, further development will proceed to testing in the prevention setting.

- **Mammoglobin cDNA vaccine**

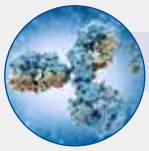
  William Gillanders

  Mammoglobin-A, a member of the secretoglobin superfamily, is a novel breast cancer-associated antigen and an exceptional target for breast cancer vaccine therapy. With support from a BCRP Clinical Translational Research Award, a Phase I clinical trial of a mammoglobin-A cDNA vaccine has been completed. The results of the trial showed that the mammoglobin-A vaccine is safe, able to induce specific IFN-γ-secreting CD8 T cells, and results in longer progression-free survival for patients. Based on the success of that trial and with support from a BCRP Breakthrough Level 3 Award, a Phase Ib trial of the mammoglobin-A DNA vaccine in patients receiving neoadjuvant endocrine therapy is underway.

- **E75 Her2-derived Peptide Vaccine (NeuVax™)**

  Constantin Ioannides, Elizabeth Mittendorf

  The BCRP supported a study that sought to identify cytotoxic lymphocyte-recognized epitopes on HER2-overexpressing human breast tumors, during which Dr. Ioannides, together with Dr. Bryan Fisk, discovered E75, an immunodominant HER2 peptide. The E75 peptide combined with GM-CSF has since been developed into an immunogenic peptide-based vaccine under the commercial name of NeuVax™ (Galena Biopharma). The vaccine reportedly targets 50-60% of HER2+ patients. NeuVax™ is now in Phase III clinical trials to evaluate the effectiveness of the vaccine to prevent or delay breast cancer recurrence after standard of care therapy. Also supported by the BCRP is a newly opened Phase II clinical trial, led by Dr. Elizabeth Mittendorf, testing NeuVax™ and trastuzumab in high-risk HER2+ breast cancer patients. In the Phase II trial, NeuVax™ will be given in the adjuvant setting to prevent recurrences in patients who were administered neoadjuvant chemotherapy, plus trastuzumab, and did not have a pathologically complete response.



Immunotherapies:

- **HER2 Bi-Armed Activated T Cells**
  Lawrence G. Lum
  The BCRP supported preclinical studies on HER2 bi-armed activated T cells, which induce the development of "memory" antigen-specific cytotoxic T cells directed at HER2. This led to a Phase I clinical trial in women with HER2+ metastatic breast cancer. Trial results indicated safety and long-term antitumor responses. A Phase II trial is ongoing.

- **TRC105**
  Ben Seon
  The BCRP supported the development of TRC105, a monoclonal antibody that targets endoglin, inhibits angiogenesis, and was found to suppress the growth of both established tumors and new tumors in preclinical models. The antibody is currently in a Phase I clinical trial, in combination with capecitabine, in breast cancer patients, as well in as several other early-phase clinical trials in other cancers.

- **Tumor Antigen-Targeted T-Cell Therapy for Metastatic Breast Cancer**
  Michel Sadelain, Shanu Modi
  The use of engineered T cells in adoptive cell therapy is a promising strategy to establish tumor immunity and eradicate tumor burden. The BCRP is supporting a Phase I clinical trial, led by Dr. Michel Sadelain and Dr. Shanu Modi at the Memorial Sloan Kettering Cancer Center, of adoptive T-cell therapy in metastatic TNBC patients. This trial is based on preclinical work that demonstrated mesothelin (MSLN) expression in 36% of TNBC patients and found that those MSLN-positive TNBC patients had an increased frequency and interval to develop distant metastases, resulting in a significantly lower overall and disease-specific survival. The goal of the trial is to systemically administer MSLN-targeted chimeric antigen receptor (CAR) T cells in patients with therapy-refractory, metastatic TNBC, and compare immune responses between patients who received MSLN CAR T cells, either intravenously or intrapleurally. The Phase I trial for intrapleural delivery has been initiated, and the intravenous trial is anticipated to begin by 2017.

Novel Techniques in Treatment:

- **HER2-Targeted Drug Delivery**
  John Park, James Marks
  The BCRP supported development of an anti-HER2/neu monoclonal antibody (MM-302) that efficiently targets HER2-overexpressing breast cancer cells. MM-302 has been licensed by Merrimack Pharmaceuticals and is now in a multiinstitutional Phase II trial involving patients with advanced stage HER2-positive breast cancer.

- **Targeted HER2 Radiotracer**
  Gary Ulaner
  Growing evidence suggests that HER2 expression may change between primary HER2- lesions and HER2+ metastases, an example of tumor heterogeneity. Currently underway is a Phase I trial using a targeted HER2 radiotracer (89Zr-trastuzumab) to determine the proportion of patients with HER2-negative primary breast cancer who develop imagable HER2-positive metastases. The trial will also determine whether HER2-targeted therapy results in a measurable treatment response.

- **Polycationic Peptides for Fluorescence-Guided Surgery**
  Roger Tsien
  With support from the BCRP, Dr. Roger Tsien, a 2008 Nobel Laureate, laid the foundation for clinical trials of imaging and therapeutic agents, based on the science of cell-penetrating polycationic peptides and their ability to transport dyes and therapeutic cargo. Dr. Tsien built a second-generation intraoperative surgical system that can simultaneously image two different fluorophores, along with a reflectance image. Currently, an in vivo fluorescent protease-activatable peptide, AVB-620, that detects, marks, and visualizes cancer has been licensed by Avelas Biosciences and is in the final stages of a Phase Ib clinical trial in breast cancer. AVB-620, given to patients prior to surgery, will help surgeons make medical decisions in real-time by identifying critical cancer margins for tumor resection and examining lymph nodes for invasive disease.

Therapeutics:

- **Center of Excellence for the Eradication of Brain Metastasis**

  Patricia Steeg

  This BCRP Center of Excellence (COE) Award formed the first group to examine brain metastasis of breast cancer in a comprehensive multi-disciplinary manner. A Phase I trial based on the group's preclinical work using temozolomide, pazopanib, and lapatinib is currently being designed. The group has concept approval to open a temozolomide secondary brain metastasis prevention trial at the National Cancer Institute (NCI).

- **IDO Inhibitor**

  George C. Prendergast

  Indoleamine 2,3 dioxygenase (IDO) is an enzyme commonly activated in breast cancer. As a result of BCRP-funded pre-clinical studies, the D isomer of an IDO inhibitor (D-1MT) is now in clinical trials for breast cancer and other solid tumors.

- **Combining Aromatase and Src Inhibitors**

  Joyce Slingerland, Isabel Chu

  BCRP-funded studies found that a two-pronged approach to therapy that includes both anti-estrogens and drugs that preserve p27 may be effective in arresting cell cycle progression in breast cancer. Phase I and II trials have begun to test the tolerability and efficacy of anastrozole, an aromatase inhibitor that stops estrogen production, together with Src inhibitor AZD0530, in post-menopausal women with ER+ breast cancer.

- **5-Fluoro-2'deoxycytidine (FdCyd)**

  Edward Newman

  Preclinical studies demonstrated the effects of FdCyd with tetrahydrouridine on reversal of DNA methylation in several genes expressed by breast cancer cells. A BCRP-funded Phase I trial has been completed, and an NCI-supported Phase II trial has been initiated.

- **Anti-Androgen Therapy (Enzalutamide)**

  Anthony Elias, Jennifer Richer

  The BCRP supported several discoveries that revealed a critical role of androgen as a driver of breast cancer growth. Higher levels of androgen receptor were found to be expressed on ER+ breast cancers that are resistant to anti-estrogen therapy. Such cells were shown to proliferate in response to androgen, and this effect was inhibited by enzalutamide. These preclinical results led to current Phase I and II clinical trials to test the safety and efficacy of enzalutamide in breast cancer, in combination with other standard treatments.

- **Meclofenamate for Brain Metastasis.**

  Joan Massague

  The BCRP funded work that created the first mouse models of latent metastasis of breast cancer. This work identified a carcinoma-astrocyte gap junction as a mechanism for metastatic outgrowth that can be inhibited by gap junction modulators such as Meclofenamate, an FDA-approved non-aspirin non-steroidal anti-inflammatory drug. As a result, a Phase I clinical trial of Meclofenamate is currently enrolling patients with recurrent or progressive brain metastasis from a solid primary tumor (NCT 02429570).

- **Aspirin as Adjuvant Therapy for Node-Positive Breast Cancer**

  Eric Winer, Michelle Holmes

  Epidemiological and preclinical data suggest aspirin may reduce breast cancer recurrence and improve survival. A BCRP-supported Phase III randomized, placebo-controlled trial of aspirin among breast cancer patients with node-positive disease is set to begin in 2017. Using invasive disease-free survival as the primary end point, the trial will assess adherence to and toxicity of long-term aspirin use, as well as create a longitudinal biospecimen and epidemiologic data repository.

- **Pembrolizumab and Tremelimumab for Treatment of Oligometastasis**

  Andy Minn

  BCRP-funded studies investigated how previously identified gene programs promote metastasis or treatment resistance; how these programs are regulated; and how oligometastases can be effectively treated. Based on this pre-clinical work, Dr. Minn and colleagues have opened a Phase I clinical trial, with assistance from Merck, that examines radiation to a metastatic lesion in combination with the immune checkpoint inhibitor pembrolizumab (PD-1 inhibitor) for patients with metastatic cancers for which anti-PD-1 therapy has failed, or for patients who have progressed after at least one regimen of systemic therapy (https://clinicaltrials.gov/ct2/show/NCT02303990). A second Phase I trial has also started to test radiation in combination with dual immune checkpoint blockade, using tremelimumab (anti-CTLA-4) and MED14736 (anti-PDL1) for patients with metastatic breast, and other cancers (https://clinicaltrials.gov/ct2/show/NCT2639026).

Diagnostics:

- **Intraductal Techniques**
  Susan Love
  Dr. Love modified an endoscope to enter and examine milk ducts through their openings at the nipple. This laid the groundwork for the development of sophisticated and miniaturized endoscopes that enable the retrieval of cell samples for detailed study of the breast ducts, which are believed to be the site where most breast tumors initiate.

- **Skp2 Oncogene**
  Michele Pagano
  High Skp2 expression correlating with destabilization of p27 is associated with poor prognosis in breast cancer patients. These findings contributed to the use of Skp2/p27 immunohistochemical analysis as a prognotic test performed in clinical pathology laboratories. Skp2 is currently being assessed as an in vivo diagnostic test for breast cancer.

Defining the Role of Nanoscale Membrane Bridges in Metastatic Breast Cancer

Shiladitya Sengupta, Ph.D., Brigham and Women's Hospital

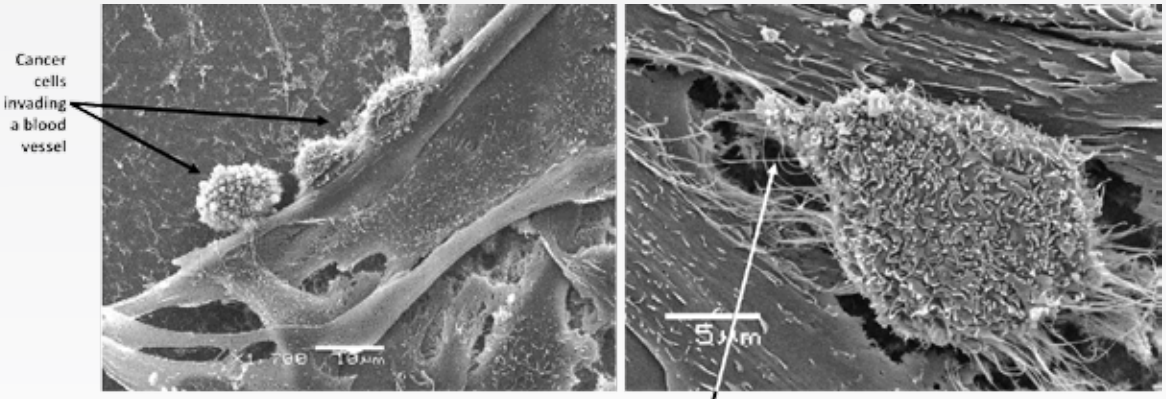
An in-depth analysis of communication between tumor cells and vascular endothelium in the context of metastasis, primarily during extravasation, is greatly needed. Nanoscale intercellular membrane bridges act as physical conduits for transferring miRNAs, which is thought to aid in the process of metastasis. By targeting these membrane bridges through pharmacological inhibition, new treatment methods may be possible for breast cancer patients.

With funding from an FY13 BCRP Breakthrough Award, Dr. Shiladitya Sengupta is exploring the understudied area of communication between tumor cells and endothelium. In a recent publication in *Nature Communications*, Dr. Sengupta has shown the preferential formation of nanoscale intercellular membrane bridges between metastatic cancer cells and endothelial cells. These bridges, which facilitate an invasive phenotype, were found to be composed of actin supported by tubulin cytoskeletal components. Through an evaluation of the kinetics of formation, Dr. Sengupta showed that the structures occurred in a directed manner, pointing to a functional role. These bridges also act as conduits through which miRNAs can be transferred from the cancer cells to the endothelium. The ability to form nanoscale conduits with endothelial cells was found to correlate with the metastatic potential of the cancer cell. Dr. Sengupta also investigated the pharmacological effects of docetaxel combined with latrunculin A or cytochalasin D. Both drug combinations disrupted the formation of heterotypic intercellular nanostructures without inducing anti-mitotic effects leading to cell death. Using a mouse model of breast cancer metastasis, Dr. Sengupta further showed that pharmacological intervention with these drug combinations resulted in reduced metastasis.

Dr. Sengupta's research provides a foundation for a novel paradigm in which reducing metastatic burden may be achieved by targeting nanoscale intercellular membrane bridges. This work could highlight new targeted therapies in treating metastatic breast cancer.

Publication:

Connor Y, Tekleab S, Nandakumar S, et al. 2015. *Nature Communications* 6:8671.



Metastatic cancer cells form nanoscale bridges with endothelial cells lining blood vessels and inject miRNA through these nanobridges to control the endothelial cells.

New Approaches to Eradicate Aggressive Breast Cancers

Andrei Goga, M.D., Ph.D., University of California, San Francisco

TNBC is a particularly aggressive subtype of breast cancer that has no targeted treatment. It has recently been discovered that the oncogene, MYC, is elevated in TNBC, opening up promising opportunities for the development of new targeted therapeutic strategies that will allow selective killing of MYC-overexpressing TNBC cells.

With support from an FY11 Era of Hope Scholar Award, Dr. Andrei Goga has taken a multi-faceted approach to identifying new therapeutic targets in MYC-driven TNBCs. In the first part of the study, Dr. Goga's team isolated disseminated tumor cells (DTCs) from patient-derived xenograft models of breast cancer. They found that metastatic cells from tissues with a low tumor burden had enhanced stem cell-like gene signatures, while those tissues with a high tumor burden displayed signatures closer to that of the primary tumor. Moreover, cells from tissues with a high tumor burden expressed an elevated level of MYC. As such, the DTCs from tissues with a high tumor burden proved to be sensitive to the cyclin-dependent kinase inhibitor dinaciclib (Merck). After a four-week treatment course, DTCs were found in only 1 of 24 drug-treated mice, compared to 11 of 25 vehicle-treated mice. However, many animals still had significant primary tumors at the end point of the study, suggesting that the inhibitory effects of dinaciclib were greater on metastatic tumors than on primary tumors. This is an important finding, as clinical trials of dinaciclib for the treatment of various cancers are already underway.

In the second part of the study, using a targeted metabolomics approach, Dr. Goga's team identified fatty acid oxidation (FAO) intermediates as being significantly upregulated in a MYC-driven model of TNBC. This represents one of the first studies to investigate the role of MYC in the metabolism of TNBC in vivo, and the results warrant further investigation into the inhibition of FAO as a therapeutic strategy for TNBC patients.

Publications:

Lawson DA, Bhakta NR, Kessenbrock K, et al. 2015. *Nature* 526(7571):131-135.
Camarda R, Zhou A, Kohnz R, et al. 2016. *Nature Medicine* epub ahead of print (doi: 10.1038/nm.4055).

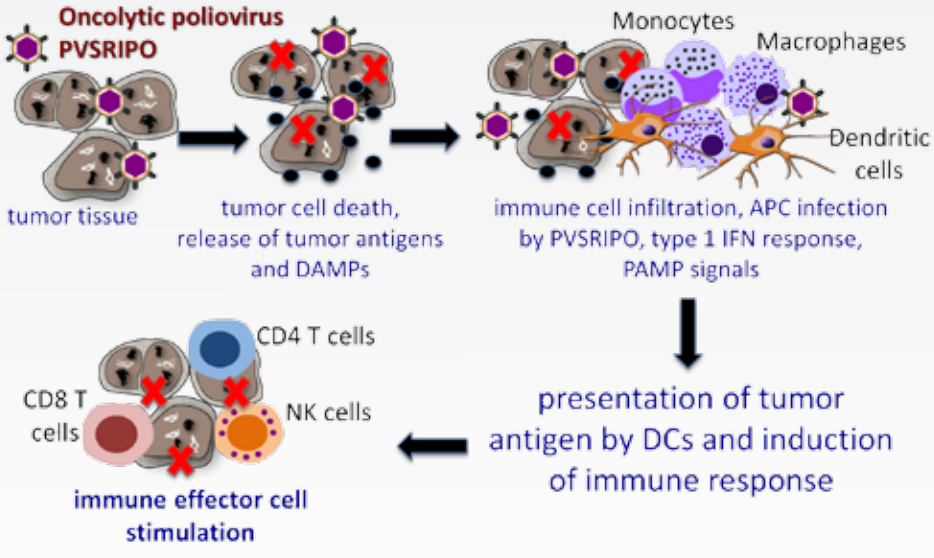
Regional Oncolytic Poliovirus Immunotherapy for Breast Cancer

Smita K. Nair, Ph.D., and Matthias Gromeier, Ph.D., Duke University

In the March 29, 2015, episode of *60 Minutes* titled, "Killing Cancer," researchers and clinicians at Duke University presented exciting results on the use of a modified poliovirus as an oncolytic immunotherapy. With support from a DoD BCRP 2003 Concept Award (BC033115), Dr. Matthias Gromeier, a molecular biologist at Duke University, established that this approach is suitable, in principle, for breast cancer as well. The oncolytic therapy functions by targeting the poliovirus receptor, CD155, which is aberrantly expressed in most cancer cells. Due to CD155 overexpression in brain cancers, as described in the *60 Minutes* episode, the first clinical trial using the oncolytic poliovirus (PVSRIPO) as an immunotherapy was done in patients suffering from an aggressive brain tumor called glioblastoma. Of the 22 patients that participated in the Phase I clinical trial, at least three showed remarkable results and are considered cancer-free, a diagnosis rarely seen in patients with recurrent glioblastoma.

Considering these groundbreaking results in glioblastoma, the PVSRIPO oncolytic immunotherapy could have an even greater impact, since it is expected to have utility in a diverse range of tumors expressing CD155, including breast cancer. To move this promising vaccine into breast cancer clinical trials, however, rigorous preclinical studies demonstrating efficacy must be performed to obtain approval by the FDA as an IND for breast cancer. Dr. Smita Nair, an immunologist interested in translational research in the Department of Surgery at Duke University and a colleague of Dr. Gromeier, received an FY15 BCRP Breakthrough Award Funding Level 3 Award to assist in achieving FDA approval to allow for Phase I trials of PVSRIPO oncolytic immunotherapy in TNBC patients. Immune cell activation and infiltration into tumors can activate adaptive immune resistance by the upregulation of the programmed death molecule 1 ligand (PD-L1). Therefore, Dr. Nair has proposed to combine PD-L1 inhibitors with PVSRIPO in order to eliminate adaptive resistance and potentiate a durable antitumor immune response. They plan to establish PVSRIPO efficacy in an immunocompetent breast cancer mouse model and investigate whether blocking PD-L1 augments therapeutic response. If PVSRIPO proves to be bioactive and effective in the preclinical experiments, the team will pursue a pilot clinical study of intratumoral injection in women with locally recurrent triple-negative breast cancer. If successful, this treatment combination will contribute to a pathway toward eradicating the mortality associated with metastatic breast cancer.

Publication:
Holl EK, Brown MC, Boczkowski D, et al. 2016. *Oncotarget* 7:79828-79841.



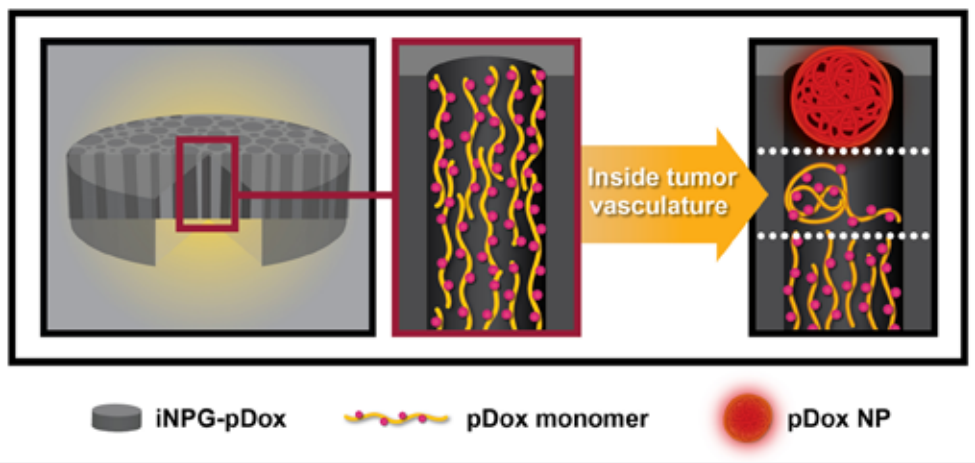
DAMPS and PAMPS: danger- and pathogen-associated molecular patterns
APC: antigen-presenting cells, such as dendritic cells (DCs)

An Injectable Nanoparticle Generator Enhances Delivery of Cancer Therapeutics

Mauro Ferrari, Ph.D., The Methodist Hospital Research Institute

Numerous biological barriers prevent adequate drug delivery and accumulation within tumors, thwarting effective tumor eradication. As a result, many scientists have focused their research on creating drug carriers with the ability to bypass these biological barriers and allow for proper drug delivery to primary and metastatic lesions. To improve the utility of nanoparticles for drug delivery, Dr. Mauro Ferrari, supported by an FY11 BCRP Innovator Expansion Award, explored the use of an injectable nanoparticle generator (iNPG) created by his laboratory that was loaded with polymeric Doxorubicin (pDox) to treat metastatic lung tumors in mice.

In a landmark paper published in *Nature Biotechnology*, Dr. Ferrari was able to selectively inhibit metastatic lung tumor progression in a mouse model of human breast cancer and effectively cure a subset of treated mice. The designed iNPG consisted of a nanoporous silicon particle packaged with pDox (iNPG-pDox) that had the ability to self-assemble into nanoparticles within the tumor microenvironment and be released intratumorally. Using multidrug-resistant MDA-BM-231 breast cancer cell lines and their non-resistant counterparts in vivo, Dr. Ferrari showed, for the first time, that iNPG-pDox was able to reverse resistance to doxorubicin, leading to a reduction in lung metastatic tumor load, whereas the individual nanoparticle components or current therapeutic formulations used as controls had no effect on the lung tumor burden. The treatment significantly improved the long-term survival of mice with lung metastases and effectively cured approximately 40% of those animals. Using the iNPG-pDox, Dr. Ferrari effectively inhibited lung metastatic progression and, moreover, eliminated toxicity to cardiac tissue. Houston Methodist Hospital has developed Good Manufacturing Practices for the iNPG-pDox and plans to fast-track the research to obtain FDA approval. Safety and efficacy studies in humans are planned to commence in 2017.



Publication:

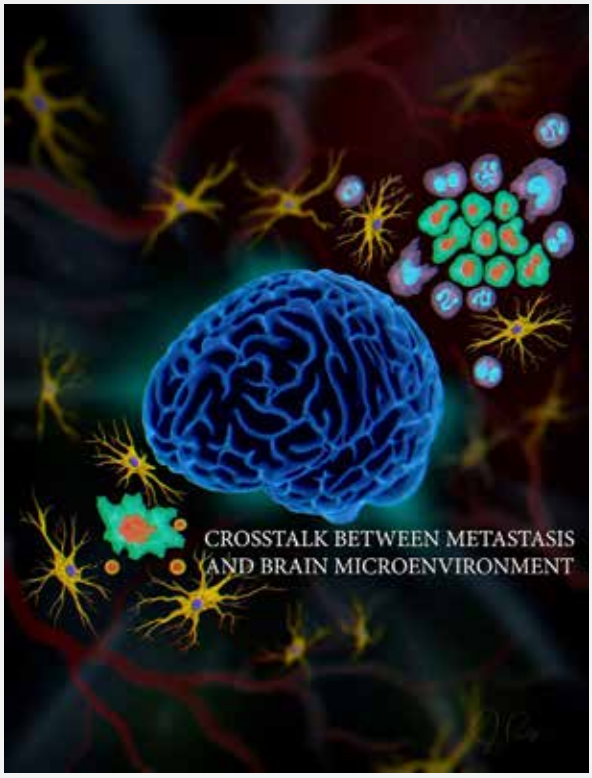
Xu R, Zhang G, Mai J, et al. 2016. *Nature Biotechnology* 34(4):414-418. doi:10.1038/nbt.3506.

Defining the Critical Role of Brain Metastases in Breast Cancer

Dihua Yu, M.D., Ph.D., M.D. Anderson Cancer Center, University of Texas

Brain metastasis is one of the major hurdles for treating metastatic breast cancer patients and occurs in 10% to 16% of metastatic cases. Following diagnosis of brain metastasis, there is only a 20% survival rate over 1 year. In FY05, the BCRP funded a Breast Cancer CoE Award led by Dr. Patricia Steeg. This collaboration is a community of consumers and investigators with the goal of eradicating brain metastases in breast cancer. Dr. Dihua Yu, a co-investigator on the CoE award, contributed her expertise in the molecular biology of metastatic aggressiveness, as well as animal models.

In November 2015, Dr. Yu, Dr. Steeg, and their CoE colleagues published a paper in *Nature* on how the loss of the tumor suppressor, PTEN, influenced by the brain microenvironment and mediated by exosomal miRNA, primes outgrowth of brain metastasis. Results showed that PTEN-normal primary tumor cells lose their PTEN expression after dissemination to the brain, but no other organs. After exiting the brain microenvironment, the tumor cells were found to regain PTEN levels. Exosomes from astrocytes were found to mediate intercellular transfer of miRNAs into the metastatic tumor cells, targeting PTEN expression. Blocking exosome secretion rescued the PTEN loss and suppressed brain metastasis. This adaptive PTEN loss was found to increase secretion of a cytokine, CCL2, which recruits myeloid cells that enhance outgrowth of brain metastatic tumor cells. Targeting CCL2 is a potential novel clinical application in treating life-threatening brain metastases. Overall, this research revealed a complex reciprocal communication between metastatic tumor cells and the tumor microenvironment in the brain. This underscores how the microenvironment may influence the outgrowth of cancer cells to form lethal metastases.



Publication:

Zhang L, Zhang S, Yao J, et al. 2015. *Nature* 527(7576):100-104. doi: 10.1038/nature15376.

Role of a Novel Transcriptional Co-Regulator in Mammary Gland Development and Breast Cancer

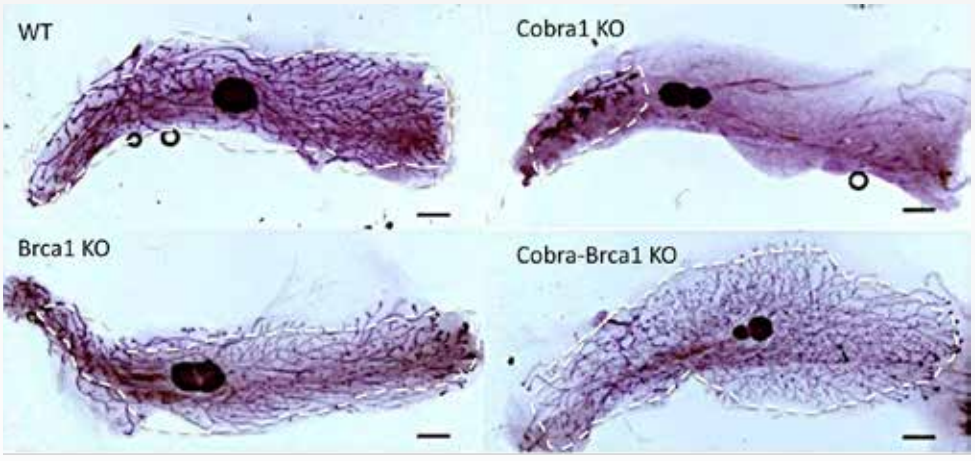
Sreejith Janardhanan Nair, Ph.D., University of Texas Health Science Center at San Antonio

Previous research identified aberrant breast cancer 1 (*BRCA1*) gene expression in women as a major risk factor for breast cancer development. BRCA1 also interacts with a complement of other genes that may impact the development of normal or cancerous breast tissue. Dr. Nair, supported by an FY08 Predoctoral Traineeship Award, and his mentor, Dr. Rong Li, determined that one BRCA1-binding protein, a cofactor of BRCA1 (COBRA1), which suppresses the growth of ER-alpha-positive breast cancer cells, plays a critical role in the development and function of adult mouse mammary glands. These findings were recently documented and expanded upon in a *Nature Communications* article.

In this publication, Dr. Nair reported using mouse models harboring mammary tissue-specific deletion of *COBRA1* or both *COBRA1* and *BRCA1* to investigate the interplay of these two genes during mammary tissue development. He found that lack of *COBRA1* in this tissue caused developmental defects in several mammary gland epithelial cell compartments, mammary ducts, alveolar tissue that houses milk-secreting cells, and milk proteins, suggesting that *COBRA1* plays an integral role in adult breast tissue development. Interestingly, simultaneous elimination of COBRA1 and BRCA1 expression in mouse mammary tissue rescued the development and function of mammary glands. When Dr. Nair delved into the mechanism behind these results, he found that COBRA1 was important for the transcription of several key adult mammary tissue developmental genes expressed during puberty and that BRCA1 may antagonize this COBRA1-dependent pubescent gene expression. These findings implicate *BRCA1* as a genetic complement of *COBRA1* in the physiological development of mammary glands and the maintenance of mammary tissue homeostasis. Furthermore, the group found that primary tumors from patients with metastatic or local recurrence had significantly lower levels of *COBRA1* mRNA expression than primary tumors of patients whose breast cancer did not recur. The laboratory is currently exploring the effect of the interaction between COBRA1 and BRCA1 on the functional status of mammary stem/progenitor cell populations in order to determine the role of COBRA1 in breast cancer progression.

Publication:

Nair SJ, Zhang X, Chiang HC, et al. 2016. *Nature Communications* 7:10913.



Whole mount of mammary gland from indicated genotypes showing growth defects in mammary ducts (outlined with white dashed lines) are rescued by co-deletion of *BRCA1*

Identifying Compounds for Targeted Breast Cancer Treatment and Prevention

Richard T. Pomerantz, Ph.D., Temple University School of Medicine

DNA breakage occurs frequently within cells and must be repaired accurately through pathways such as homologous recombination (HR) to prevent the formation of cancer-causing genetic mutations. Heritable mutations in central HR genes *BRCA1* and *BRCA2* cause defects in the HR repair of DNA breaks and strongly predispose women to breast cancer. The development of drugs that preferentially kill *BRCA1/2*-deficient cancer cells, while limiting negative side effects, would have a significant impact for patients.

With support from an FY13 Breakthrough Award Level 1, Dr. Richard Pomerantz performed a high-throughput screen for small molecules that inhibit the HR activity of RAD52. Dr. Pomerantz identified 6-hydroxy-DL-dopa (6-OH-dopa), which alters the structure of RAD52, disrupting its binding to ssDNA and its ability to function in HR DNA repair. Importantly, 6-OH-dopa was found to selectively kill *BRCA1/2*-deficient cancer cells, making it a promising therapeutic agent. These studies demonstrate for the first time that RAD52 can be targeted for treatment using drugs that disrupt the ringed protein structure. Moving forward, Dr. Pomerantz will examine the potential use of 6-OH-dopa as precision medicine for *BRCA1/2*-deficient breast cancer patients and test additional compounds that inhibit RAD52 via a similar mechanism.

Publication:

Chandramouly G, McDevitt S, Sullivan K, et al. 2015. *Chemistry & Biology* 22:1491-1504.

BET Bromodomain Inhibitor Shows Promise as Triple-Negative Breast Cancer Treatment

Xiaole Shirley Liu, Ph.D., Dana-Farber Cancer Institute

Current standard-of-care treatment for TNBC is limited to aggressive chemotherapies, with no targeted therapies available. To address the critical need for new therapeutics, Dr. Xiaole Liu, with support from an FY12 Idea Award, aimed to determine the efficacy of BET bromodomain inhibitors, a class of drugs successfully used to treat other cancer types.

BET proteins are a family of chromatin-binding proteins that are implicated in promoting oncogenic transcriptional programs. The BET bromodomain inhibitor, JQ1, works by competing with the BET protein BRD4, an oncogenic protein that is highly expressed in TNBC and other cancers, for binding to chromatin. Small changes to proteins contained in chromatin can have broad genetic consequences.

Dr. Liu and her colleagues hypothesized that displacement of BRD4 from chromatin by JQ1 would interfere with the BRD4-triggered oncogenic programming in TNBC cells. As shown in a recent publication in *Nature*, she and her colleagues found that JQ1 treatment preferentially arrested growth of TNBC tumors in mouse xenograft models, as well as patient-derived xenografts, suggesting JQ1 as a potential treatment for human TNBC. While TNBC cells typically remained dependent on BRD4 binding to chromatin, in some cases, TNBC cells were able to circumvent the effects of JQ1 treatment by causing epigenetic changes that allowed BRD4 binding to chromatin in a bromodomain-independent manner. These results provide a rationale for BET inhibition in TNBC.

Publication:

Shu S, Lin C, He HH, et al. 2016. *Nature* 529(7586):413-417.

New Approach to Cancer Prevention in BRCA1 Mutation-positive Women

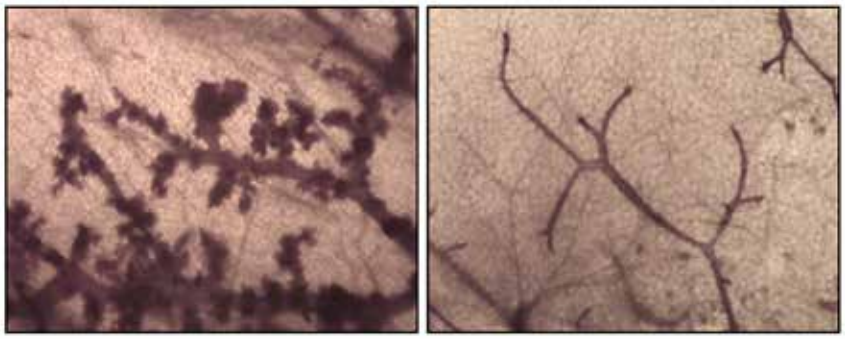
Josef Penninger, M.D., Institute of Molecular Biotechnology, Vienna, Austria

Women with inherited mutations in the *BRCA1* or *BRCA2* genes are at substantially higher risk of breast cancer. For these women, effective prevention strategies are their best hope to reduce their breast cancer risk. In FY11, Dr. Josef Penninger received an Innovator Award from the BCRP to pursue a project that could immediately impact breast cancer prevention.

Dr. Penninger's team was the first to demonstrate that two proteins, RANK and RANKL, are important regulators of bone loss, ultimately leading to FDA approval of RANKL-blocking drugs for osteoporosis. His team later discovered that the RANK/RANKL system is also essential for the formation of the lactating mammary gland during pregnancy, and that it forms a crucial molecular link between sex hormones, development of breast cancer, and subsequent metastasis to bone. Interestingly, RANK signaling was found to act on mammary epithelial progenitor cells, which are also believed to be "seed cells" for TNBC in carriers with *BRCA1/2* mutations. With his BCRP Innovator Award, Dr. Penninger investigated whether RANKL inhibition could be used to prevent breast cancer in mouse models.

Dr. Penninger's results showed that inactivation of RANK markedly delayed and, in some cases, prevented the development of breast cancer in mice with mutated *BRCA1*. The loss of RANK also impaired the progression of breast tumors to high-grade malignancies. These effects were observed both when RANK was inactivated genetically and when mice received preventive pharmacological RANKL inhibitors.

Dr. Penninger has documented that RANK/RANKL are highly expressed in pre-malignant lesions and breast cancer in women with *BRCA* mutations, and that common variants of RANK are associated with increased breast cancer risk among these women. Future experiments, especially carefully designed clinical trials, will be needed to assess whether RANKL inhibitors offer an advantage over current prevention techniques like Tamoxifen or oophorectomy. However, these findings raise the exciting possibility that inhibition of RANKL, for which there is already a drug that has a good safety record and is approved by the FDA, could offer a novel, targeted approach for breast cancer prevention in women with *BRCA1* mutations.



The figure shows mammary glands from 4-month-old mice carrying a mutation in the *BRCA1* gene. Blocking the RANKL/RANK system leads to largely normal mammary glands (right), whereas malignant changes and carcinomas could be found in the control group (left). Copyright: IMBA

Publication:

Sigl V, Owusu-Boaity K, Penninger JM, et al. 2016. *Cell Research* 26(7):761-774.

RB1 Deficiency in Triple-Negative Breast Cancer Induces Mitochondrial Protein Translation

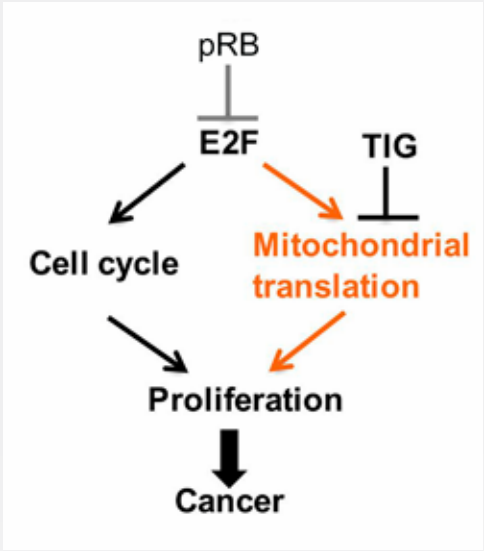
Robert A. Jones, Ph.D., University Health Network, Toronto

Dr. Robert Jones

Dr. Eldad Zacksenhaus

Chemo-resistant TNBC patients have few therapeutic options. Tumor protein 53 (p53) and Retinoblastoma 1 (RB1) are two tumor suppressor proteins that have been found to be frequently lost together in TNBC patients and are, therefore, not directly targetable; however, their downstream effectors could be. With support from an FY09 Postdoctoral Fellowship Award, Dr. Robert Jones set out to elucidate vulnerabilities that could be therapeutically exploited downstream of p53 and RB1 signaling in hopes of revolutionizing treatment of TNBC by providing a targeted therapeutic option.

In a landmark publication*, Dr. Jones, together with a large team of researchers under the guidance of Dr. Eldad Zacksenhaus, demonstrated for the first time that a combined loss of p53 and RB1 enhanced the mitochondrial protein translation (MPT) pathway, which was susceptible to an FDA-approved MPT inhibitor tigecycline (TIG). Treatment of p53/RB1-deficient TNBC cell lines with TIG resulted in reduced cellular proliferation, mitochondrial protein expression, and a tumor-initiating cancer stem cell-like cell population in an RB1-dependent manner. Among a panel of TNBC cell lines, TIG was shown to synergize with sulfasalazine, an FDA-approved drug shown to attenuate the growth of TNBC cells, to further reduce cellular proliferation.



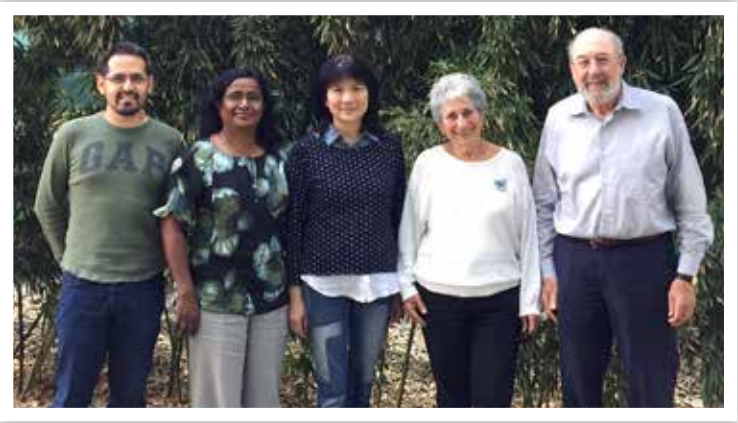
This work demonstrates that tumor vulnerabilities can be identified within TNBC tumors and can be exploited therapeutically. Dr. Jones' and his colleagues' research supports not only a role for TIG and other MPT inhibitors in the treatment of p53/RB1-deficient TNBC, but also provides insight into the biology of these aggressive tumors that could be exploited in future studies to develop other novel targeted therapeutics.

Publication:

*Jones RA, Robinson TJ, Liu JC, et al. 2016. *J Clin Invest* 126(10):3739-3757.

Tetraspanin CD81 Promotes Tumor Growth and Metastasis by Modulating the Functions of T Regulatory and Myeloid-Derived Suppressor Cells

Shoshana Levy, Ph.D., Stanford University



Dr. Shoshana Levy, second from right, and colleagues

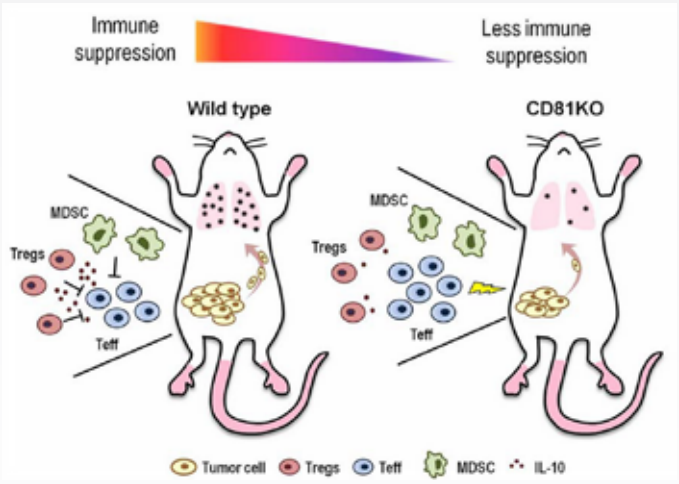
CD81, a member of the tetraspanin family of proteins, has been implicated in promoting cancer and infection. Although many studies have examined the role of CD81 in modulating immune function in response to infection, few have studied its role in tumorigenesis and metastasis. With an FY13 BCRP Breakthrough Level 2 Award, Dr. Shoshana Levy's team set out to determine whether inhibition of CD81 could prevent metastatic dissemination of breast cancer cells.

In a recent publication,* Dr. Levy described her work leading to the discovery that decreasing host expression of CD81 in mice could result in a reduction of tumor growth, progression, and lung metastases. Injection of breast cancer cells into CD81 knockout (KO) mice resulted in reduced tumor volumes and a highly significant decrease in lung metastases. In ex vivo and adoptive transfer studies, Dr. Levy and her team showed that reduced tumor volume and metastases could be attributed to inhibition of regulatory T cell (Treg) and myeloid-derived suppressor cell (MDSC) function. Tregs and MDSCs from CD81 KO mice were no longer able to inhibit antitumor immune cells, thus restoring the antitumor immune response.

Dr. Levy and her team have shown, for the first time, that host expression of CD81 drives tumor growth, progression, and metastasis through inhibition of antitumor immune responses. Therapeutics that block Treg and MDSC function could reverse tumor-induced immune suppression and ultimately help reduce the mortality associated with metastatic breast cancer.

Publication:

*Vences-Catalán F, Rajapaksa R, Srivastava MK, et al. 2015. *Cancer Res* 75(21):4517-4526.



Impaired immune suppression in CD81KO mice results in reduced tumor growth and, especially, in metastatic burden. In wild-type mice, primary tumors grow and recruit immune suppressor Tregs, and MDSCs. These suppressor cells secrete IL-10 and inhibit the antitumor T effector (Teff) response, allowing tumors to grow and metastasize to the lungs (left panel). In contrast, Tregs and MDSCs still accumulate in CD81KO mice, but are impaired at suppressing Teff cells, thereby reducing tumor growth and metastasis (right panel).

Targeting Nuclear Fibroblast Growth Factor to Improve Chemotherapy Response in Triple-Negative Breast Cancer

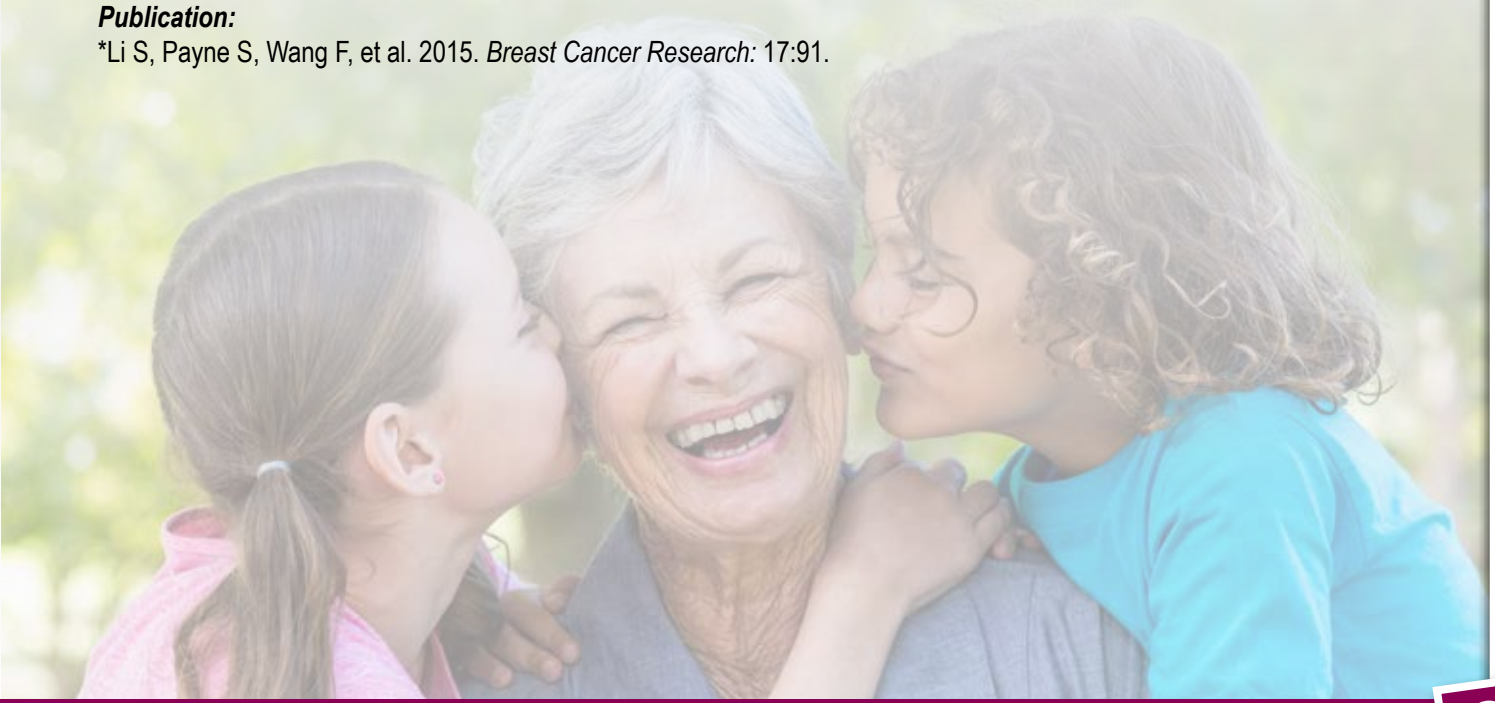
Robin Bachelder, Ph.D., Duke University

Dr. Robin Bachelder, right front, and colleagues

The aggressiveness of TNBC is largely due to its resistance to standard chemotherapies. Most TNBCs never exhibit a pathologically complete response to chemotherapy, and at least half of those patients succumb to chemo-resistant metastatic TNBC within 5 years of diagnosis. Thus far, researchers have been unable to pinpoint drivers of chemo-residual TNBC cells. Identifying such drivers would enable the development of strategies to inhibit TNBC progression post-chemotherapy. Dr. Robin Bachelder, from Duke University, observed that short-term doxorubicin treatment "enriched" the chemo-residual TNBC cell subpopulation in vitro, specifically increasing the presence of a nuclear isoform of the protein basic fibroblast growth factor (bFGF). With support from a BCRP FY12 Idea Award, Dr. Bachelder's team was able to test the hypothesis that nuclear bFGF plays a role in chemoresistance. Her team's findings, which were recently published in *Breast Cancer Research,** demonstrated that nuclear bFGF was upregulated or sustained in five of six matched TNBC cases obtained post-neoadjuvant chemotherapy treatment (compared to bFGF levels in those patients pre-chemotherapy treatment). They discovered that chemo-residual cell survival is dependent on the presence of nuclear bFGF. Gene knockdown studies identified a mechanism by which nuclear bFGF accelerates DNA repair in chemo-residual cells by upregulating DNA-dependent protein kinase (DNA-PK) activity, which helps repair double-strand breaks. Interestingly, cultures containing chemo-residual TNBC cells treated with DNA-PK inhibitors exhibited reduced cell and colony counts compared to untreated cultures. These findings show the potential of targeting this DNA repair mechanism to impede TNBC progression post-chemotherapy. Dr. Bachelder's team continues to study nuclear bFGF, bFGF/DNA-PK signaling, and the bFGF receptor to better understand its mechanism of action and potentially define a target for future TNBC therapy.

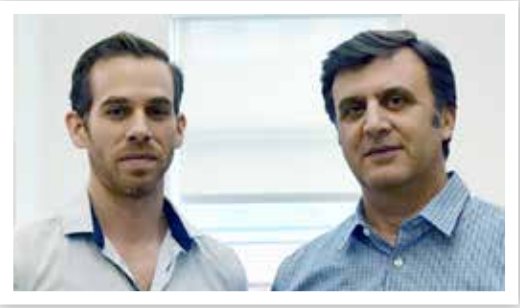
Publication:

*Li S, Payne S, Wang F, et al. 2015. *Breast Cancer Research:* 17:91.



Treatment-Induced Immune Mechanisms Associated with Tumor Dormancy and Relapse

Masoud H. Manjili, Ph.D., DVM, Virginia Commonwealth University, Massey Cancer Center



Dr. Masoud Manjili, right, and colleague

The biological and mechanistic basis for tumor dormancy and recurrence remains poorly understood. With support from an FY13 Breakthrough Award, Dr. Masoud H. Manjili is researching how antitumor immune responses induced by chemotherapy sustain tumor dormancy and how tumors may or may not be prone to immunoediting, a cellular mechanism used to evade the natural immune response.

Dr. Manjili's group at Virginia Commonwealth University, Massey Cancer Center, used a FVBN202 transgenic mouse model of breast carcinoma, along with reprogrammed T-cells and natural killer T-cells, to determine the effects of modulating cross-talk between tumors and immune cells. As shown in the *Journal of Leukocyte Biology*, after treatment with Adriamycin or radiation, remaining dormant cells became resistant to additional doses of both types of therapy, but remained sensitive to tumor-reactive immune cell therapy. Importantly, two types of dormancy were identified through this study: (1) indolent dormancy (a balance between proliferation and death) and (2) quiescent dormancy (total cessation of proliferation). They discovered that, while indolent tumor cells demonstrated immunoediting and escape, quiescent cells did not undergo immunoediting and remained prone to immunotherapy. Results also showed that reprogramming of tumor-immune cross-talk alone was effective in prolonging the survival of animals displaying experimental circulating metastatic tumor cells. The group concluded that immunotherapy used after tumor dormancy is established, but before recurrence of the disease, could be an effective treatment against quiescent dormant tumor cells. They now plan to develop combination therapies that could establish a quiescent-like dormancy in tumor cells to render them sensitive to immunotherapy.

Publication:

Payne K, Keim R, Graham L, et al. 2016. *J Leukoc Biol.* 100(3):625-35.

Uncovering the Epigenetic Origins of Triple-Negative Breast Cancer

Kornelia Polyak, M.D., Ph.D., Dana-Farber Cancer Institute, Boston, Massachusetts

Dr. Kornelia Polyak, front third from right, and colleagues

Dr. Kornelia Polyak of the Dana-Farber Cancer Institute, recipient of an FY08 BCRP Idea Award and subsequent FY13 Idea Expansion Award, has made an important discovery regarding the origin of basal-like breast cancers. Dr. Polyak discovered that luminal and basal-like breast cancers are largely regulated by epigenetic mechanisms involving key factors that play a role in "switching" one type to the other.

As reported in a recently published article in *Cell Reports*, Dr. Polyak and her team used an advanced technique called somatic cell fusion to genetically merge luminal and basal-like breast cancer cells, and they observed the cellular and molecular changes that occurred as a result of the fusion through genetic and epigenetic profiling. They determined that epigenetic changes can drive luminal breast cancer cells to "switch" into basal-like cells, and that the basal-like cell type appears to be dominant. This is consistent with previous observations that luminal breast cancer tumors often contain individual cells with basal-like characteristics, while the reverse is rarely true. Moreover, Dr. Polyak and her team found that there are many different ways for cells to epigenetically extinguish the features of luminal breast cancer, suggesting that, even within the basal-like subtype, there is a high degree of variability. They found that basal-like breast cancer cells have an overall lower level of DNA methylation than luminal cells, and luminal cells typically use DNA methylation to silence stem/basal cell markers.

The results of Dr. Polyak's team represent a major advancement in the breast cancer field, providing new mechanistic information about the role of epigenetic factors in the development and biology of basal-like breast tumors. Ultimately, these findings may lead to new therapeutic approaches for patients with TNBC, one of the deadliest forms of breast cancer, for which there are no targeted therapies available.

Publication:

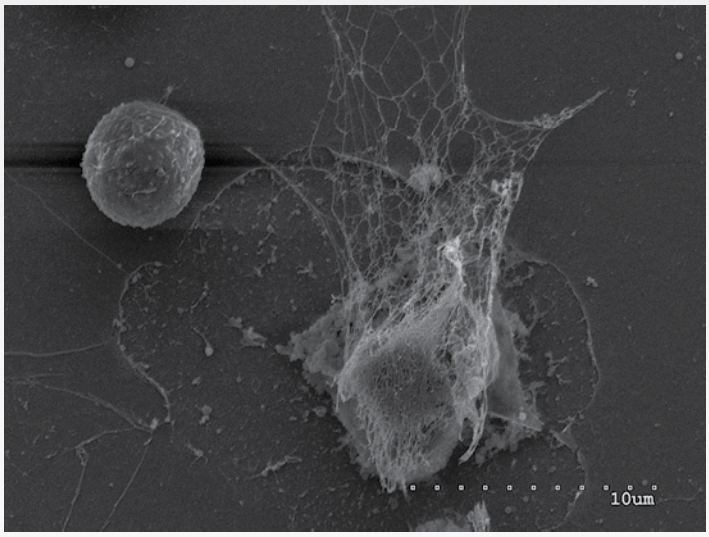
Su Y, Subedee A, Bloushtein-Qimron N, et al. 2015. *Cell Rep*. 11(10):1549-1563.

Targeting the Tumor Microenvironment to Terminate Drug-Resistant Breast Cancer

Mikala Egeblad, Ph.D., Cold Spring Harbor Laboratory

Dr. Mikala Egeblad, right front, and colleagues

One of the most challenging obstacles for treating breast cancer is the development of metastatic tumors that have acquired resistance to therapies. Studies suggest that genetic changes within a tumor cell, and more recently conditions outside of the tumor cell (called the tumor microenvironment), can influence the spread of cancer cells. Neutrophils are an integral part of the innate immune system, killing harmful microorganisms through a variety of mechanisms, including the release of DNA-protein networks, called neutrophil extracellular traps (NETs), into the microenvironment. Outside of their traditional role, NETs have also been found to accumulate in tumors with as yet unknown contributions to drug resistance and metastasis. In a study recently published in *Science Translational Medicine*, Dr. Mikala Egeblad, supported by an FY13 Era of Hope Scholar Award, demonstrated that metastatic breast cancer cells can signal neutrophils to release NETs into the tumor microenvironment in the absence of infection, promoting metastasis. NETs were also observed in tumor samples from breast cancer patients, with higher NET levels being associated with the more aggressive triple-negative subtype. Using a mouse model of breast cancer that metastasizes to the lungs, Dr. Egeblad examined the possibility of targeting NETs by treating mice with nanoparticles coated with DNase I, an enzyme that degrades DNA, and found a significant reduction in the number of breast cancer metastases. Together, these results demonstrate a novel mechanism by which tumor cells alter the inflammatory microenvironment to escape detection and promote metastases. Moving forward, Dr. Egeblad is currently further examining how NET accumulation leads to tumor progression and therapy resistance in hopes of developing targeted treatments to prevent metastatic recurrence in patients.



Publication:

Park J, Wysocki, RW, Amoozgar Z, et al. 2016. *Science Translational Medicine* 8, 361ra138.

Products Making an Impact

RESEARCH RESOURCES

Expression Arrest™ shRNA Libraries

Gregory Hannon and Stephen Elledge

RNA interference (RNAi) is a cellular system that controls which genes are active or silent. The selective effect of RNAi on specific gene expression makes it a valuable research tool. Small hairpin RNAs (shRNAs) are one of the gene silencing mechanisms of RNAi. The BCRP supported the development of whole genome shRNA libraries that target over 30,000 genes. This commercially available research tool provides researchers with ready-to-use, rapid RNAi screens for the entire human and mouse genomes to study gene regulation and identify new therapeutic targets in many diseases and conditions, including cancer.

Three-Dimensional Culture Systems

Mina Bissell

The BCRP supported the development of 3-D culture systems that have made important contributions to understanding the tissue microenvironment and how interactions between epithelial cells and the extracellular matrix control cancer development. As surrogates for in vivo studies, 3-D culture models have enabled the elucidation of oncogenic and other cell-signaling pathways that are controlled by cell-matrix interactions. 3-D culture models are currently utilized across academic laboratories and by drug companies as screening assays to test for therapeutic response.

Novel Models for Breast Tumor Growth and Metastasis

Alana Welm

Orthotopic breast tumor models can replicate the diversity of human breast cancer through patient-centric models for tumor growth, metastasis, drug efficacy, and prognosis. These models exceed the current standard of cell line xenograft models. Funding from the BCRP has supported generation of publicly available tumor graft mouse models. Since some of the most promising therapies affect the immune response and need to be tested in pre-clinical models before entering trials, work is now underway to develop immune-competent mouse models representing each subtype of human breast cancer to predict the response to therapy. Researchers interested in obtaining the models should refer to http://www.ncbi.nlm.nih.gov/pubmed/22019887. Additional unpublished models are also available by contacting Dr. Welm.

THERAPEUTICS

Herceptin®

Dennis Slamon

Herceptin® (trastuzumab) is a monoclonal antibody that targets the HER2 receptor. HER2+ breast cancer accounts for approximately 25% of all breast cancers. The BCRP was instrumental in supporting the preliminary in vitro and in vivo studies needed to test the efficacy of Herceptin®, which later led to clinical trials and commercialization. Herceptin® revolutionized breast cancer treatment and the field of targeted therapeutics. Herceptin® is now part of standard-of-care treatment regimens for HER2+ early-stage and metastatic breast cancers.

ATLAS Clinical Trial

Richard Peto

BCRP funds supported initiation of the Phase III clinical trial ATLAS (Adjuvant Tamoxifen Longer Against Shorter), the largest breast cancer treatment trial ever undertaken. Adjuvant tamoxifen is the first-line treatment for ER+ breast cancer in premenopausal women. The ATLAS trial examined whether 10 years of tamoxifen confers greater benefit than 5 years of tamoxifen. Results of the trial indicated that the risk of recurrence or death from breast cancer was reduced in women who took tamoxifen for 10 years versus 5 years.

Prone Radiotherapy

Silvia Formenti

Clinical trials were conducted to assess the efficacy of an accelerated, hypofractionated, whole-breast radiation therapy designed to minimize the length of radiotherapy required after partial mastectomy in patients with DCIS. Patients were treated in the prone position, greatly reducing unnecessary radiation exposure of the heart and lungs. Current clinical trials and long-term follow-up will continue to examine the prone radiotherapy approach for efficacy and toxicity.

Palbociclib (Ibrance®)

Dennis Slamon

BCRP-supported preclinical work on the CDK inhibitor palbociclib (Ibrance®) led to Pfizer-supported Phase I and II trials combining Ibrance® with letrozole. Results showed an increase in median progression-free survival, prompting "Breakthrough Therapy" status by the FDA and Pfizer's initiation of a Phase III clinical trial. In 2015, FDA grants accelerated approval of Ibrance® with letrozole for the treatment of ER+/HER2+ breast cancer in postmenopausal women.

Products Making an Impact

DIAGNOSTICS

Sentinel Lymph Node Biopsy

Douglas Reintgen and Kathryn Verbanac

The previous standard for detecting breast cancer invasion into the lymph nodes was to remove the majority of axillary lymph nodes and search for cancer cells. In a significant proportion of women, this causes lymphedema of the arm, a condition that compromises functionality and quality of life. In sentinel lymph node biopsy, lymph node removal is limited to only the first few nodes that receive lymph drainage from the breast. This diagnostic/ prognostic technique enables clinicians to determine tumor staging and whether more-extensive lymph node surgery is necessary. The BCRP provided funding for multicenter clinical trials to validate lymph node mapping and sentinel lymph node biopsy as an accurate method to predict the presence of disease in the axillary lymph nodes.

Molecular Breast Imaging

Carrie Hruska

Molecular breast imaging (MBI) is a nuclear medicine technique that uses high-resolution dual-head gamma cameras to detect the functional uptake of a radiotracer in the breast. MBI is an FDA-approved, commercially available technology.

Digital Mammography and Breast Tomosynthesis

Laurie Fajardo and Daniel Kopans

Digital mammography allows for an expanded detection range of x-ray signals compared to standard film mammography. The BCRP provided support to optimize this technology and to conduct a multicenter clinical validation of digital mammography. The study demonstrated that digital mammography is superior to film mammography in detecting breast cancer in women with dense breast tissue, leading to a change in clinical practice. The BCRP also supported the development and clinical evaluation of digital breast tomosynthesis. This 3-D digital mammography tool offers an additional 3-D view to capture images for improved sensitivity. A tomosynthesis system is now FDA-approved and commercialized for clinical use.

MetaSite Breast™

John Condeelis and Allison Harney

TMEM (Tumor Microenvironment of Metastasis) sites are composed of a stable interaction between three specific cells: an endothelial cell, a tumor-associated macrophage, and a MenaCalc-positive tumor cell (expressing high levels of the MenaINV protein isoform and low levels of the Mena11a isoform). In work funded by the BCRP, Drs. Condeelis and Harney found that TMEM sites are the only doorway for tumor cell entry into blood vessels. In other studies, TMEM were found in all primary and secondary sites and in all stages of breast cancer progression, making TMEM the common dissemination marker in all breast tumors and their associated distant sites. In collaboration with MetaStat, Inc., Dr. Condeelis and colleagues clinically validated the MetaSite Breast™ test, which measures TMEM levels to predict the metastatic potential of the primary tumor. MetaSite Breast™ has been licensed to MetaStat, Inc., and is CLIA (Clinical Laboratory Improvement Amendments)–certified and publically available.

MenaCalc™

John Condeelis, Jeanine Pignatelli

Breast cancer cells enter the bloodstream at sites called TMEM and spread elsewhere in the body. TMEM sites are correlated with low levels of Mena11a (MenaCalc™). A prospective clinical trial supported by the BCRP demonstrated that the MenaCalc™ score in fine needle biopsies predicted the TMEM score (i.e., a high number of TMEM sites) in resected primary breast tumor tissue. In addition, two retrospective trials showed that the MenaCalc™ score can be used as a prognostic marker for distant recurrence. MenaCalc™ has been licensed to MetaStat, Inc., and has been clinically validated for use in breast cancer treatment decision-making. It has also been used for other types of cancers, including early-stage non-small cell lung carcinoma, as an independent prognostic factor and predictor of metastasis.

PATIENT RESOURCES AND REGISTRIES

BreastCancerTrials.org

Laura Esserman

Breast cancer patients can benefit from objective information about clinical trials. The process of identifying an appropriate clinical trial by performing independent research is challenging. BCRP funding contributed to the development of an online resource (BreastCancerTrials.org) that educates patients about breast cancer clinical trials and matches them with appropriate trials.

Carolina Mammography Registry

Bonnie Yankaskas

The Carolina Mammography Registry was first funded by a BCRP award to create the infrastructure for a population-based mammography registry in North Carolina, focusing on a largely rural population. The registry became a member site of the National Cancer Institute Breast Cancer Surveillance Consortium, a national registry that provides a resource for researchers to study mammography screening on a national level.

Margaret Dyson Family Risk Assessment Program

Mary Daly

The BCRP supported the establishment of a high-risk breast cancer registry to gather genetic and environmental risk information about women with familial breast cancer. This registry evolved into the Margaret Dyson Family Risk Assessment Program for individuals at risk for breast or ovarian cancers. This program, which serves Philadelphia and its surrounding communities, provides a range of risk assessment, screening, and preventive services.

BrainMetsBC.org

Patricia Steeg

Breast cancer advocates on this team-based award led the efforts to develop an online resource (BrainMetsBC.org) that provides the latest information about brain metastases. The web site, which is available in English and Spanish, includes updates on current research, treatments, and clinical trials, as well as personal experiences written by patients.

RISK ASSESSMENT

BRCA2 617delT mutation

David Goldgar and Susan Neuhausen

Breast cancer and ovarian cancer risk is greater in individuals with mutations in the *BRCA1* and *BRCA2* tumor suppressor genes. The likelihood of *BRCA1* or *BRCA2* mutations is higher in certain populations, including individuals of Ashkenazi Jewish descent. BCRP funding contributed to the discovery of the *BRCA2* 617delT mutation, one of the three founder *BRCA1/2* mutations that occur in Ashkenazi Jews. The *BRCA2* 617delT mutation is now part of a commercialized test for *BRCA1/BRCA2* gene mutations in this risk group.

OncoVue®

Eldon Jupe

Risk association studies funded by the BCRP formed the foundation for a breast cancer risk assessment test. Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered. OncoVue® is the first genetic-based breast cancer risk test that incorporates a woman's SNPs with personal history to estimate her risk for breast cancer. This test can identify high-risk patients and enable clinicians to individualize breast cancer screening and monitoring. OncoVue® is commercially available and is currently offered at more than 30 breast care centers in the United States.

PTEN

Michael Wigler

BCRP funding contributed to the original discovery of the PTEN (phosphatase and ten- sin homolog) gene. In normal cells, PTEN functions as a tumor suppressor protein that helps regulate cell division and growth. PTEN is one of the most frequently mutated genes in several cancers, and PTEN mutations have been shown to be predictive of aggressive cancer. PTEN mutations also occur in a group of genetic syndromes that are characterized by malignant and benign tumors. A PTEN test is commercially available to confirm PTEN mutations for clinical and prenatal diagnoses and identification of at-risk family members.

PALB2 Mutations

Bing Xia

BCRP funding contributed to the discovery of PALB2, a *BRCA2* binding protein. PALB2 and *BRCA2* work together to mend broken strands of DNA, which helps to maintain the rate of cell growth. While *BRCA1* and *BRCA2* gene mutations are high-risk factors for breast cancer, these mutations do not account for all familial breast cancers. Identification of mutations in the PALB2 gene indicates an approximate twofold increase in breast cancer susceptibility due to its inability to interact with *BRCA2*. A commercialized PALB2 genetic test is available for those with familial breast cancer.

BROCA Cancer Risk Panel

Tomas Walsh and Mary-Claire King

An estimated 70% of families with multiple cases of breast cancers have no known gene mutations that increase their risk to the disease. Dr. Walsh, in collaboration with Dr. King, identified and validated rare mutations termed copy-number variants, which led to development of a comprehensive test named "BROCA" that enables assessment of all known breast cancer genes and all mutation types in a single assay. The BROCA test is currently available through the University of Washington by physician request.

PROGNOSTICS

Breast Cancer Index

Dennis Sgroi

Women with ER+ breast cancer have an increased risk of relapse many years after their initial diagnosis. To identify women with an increased risk of disease recurrence, Dr. Sgroi validated biomarkers that correlated with relapse-free survival and tumor grade, leading to a risk assessment test termed the Breast Cancer Index (BCI). The BCI test, which is now commercially available through bioTheranostics, provides a quantitative assessment of the likelihood of early and late recurrence, as well as benefit from extended endocrine therapy.

For more information, please visit
http://cdmrp.army.mil
or contact us at:
usarmy.detrick.medcom-cdmrp.mbx.cdmrp-public-affairs@mail.mil
(301) 619-7071

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